

# Three new species of *Conidiobolus* sensu stricto from plant debris in eastern China

Yong Nie<sup>1,2</sup>, Yue Cai<sup>3</sup>, Yang Gao<sup>4</sup>, De-Shui Yu<sup>1</sup>,  
Zi-Min Wang<sup>2</sup>, Xiao-Yong Liu<sup>5</sup>, Bo Huang<sup>1</sup>

**1** Anhui Provincial Key Laboratory for Microbial Pest Control, Anhui Agricultural University, Hefei 230036, China **2** School of Civil Engineering and Architecture, Anhui University of Technology, Ma'anshan 243002, China **3** Department of Biological and Environmental Engineering, Hefei University, Hefei 230601, China **4** Bioengineering and Technological Research Centre for Edible and Medicinal Fungi, Jiangxi Agricultural University, Nanchang, 330045, China **5** State Key Laboratory of Mycology, Institute of Microbiology, Chinese Academy of Sciences, Beijing 100101, China

Corresponding author: Bo Huang ([bhuang@ahau.edu.cn](mailto:bhuang@ahau.edu.cn)); Xiao-Yong Liu ([liuxiaoyong@im.ac.cn](mailto:liuxiaoyong@im.ac.cn))

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## Abstract

The genus *Conidiobolus* Bref. is widely distributed and the *Conidiobolus* sensu lato contained three other genera, *Capillidium*, *Microconidiobolus* and *Neoconidiobolus*. A molecular phylogeny based on the nuclear large subunit of rDNA (nucLSU), the mitochondrial small subunit of rDNA (mtSSU) and the translation elongation factor 1-alpha gene (TEF1) revealed three novel species within the clade of *Conidiobolus* s.s., i.e. *C. bifurcatus* **sp. nov.**, *C. taihushanensis* **sp. nov.** and *C. variabilis* **sp. nov.** These three species were isolated from plant debris in eastern China. Morphologically, *C. bifurcatus* **sp. nov.** is characterised by its secondary conidiophores often branched at the tip to form two short stipes each bearing a secondary conidium. *C. taihushanensis* **sp. nov.** is different from the others in its straight apical mycelia and the production of 2–5 conidia. *C. variabilis* **sp. nov.** is distinctive because of its various shapes of primary conidia. All these three new taxa are illustrated herein with an update key to the species of the genus *Conidiobolus* s.s.

## Keywords

basal fungi, *Entomophthorales*, taxonomy, molecular phylogenetics, new species



## Introduction

The genus *Conidiobolus* Bref. (Ancylistaceae) was established to accommodate the type *C. utriculosus* Bref. and a second species *C. minor* Bref. (Brefeld 1884). This genus was characterised by simple conidiophores, globose to pyriform conidia and resting spores formed in the axis of the hypha (mostly as zygospores) (Humber 1997). Until 1968, a total of 41 species occurring saprotrophically in soil and plant debris had been assigned to this genus (Martin 1925, Couch 1939, Drechsler 1952, 1953a, b, 1954, 1955a, b, c, 1956, 1957a, b, c, 1960, 1961, 1962, 1965, Srinivasan and Thirumalachar 1961, 1962a, b, 1965, 1967, 1968a, b). In a review of these taxa with the numerical technique, 27 definitive species were recognised (King 1976a, b, 1977). On the basis of the shape of secondary conidia, Ben-Ze'ev and Kenneth (1982) classified the genus *Conidiobolus* into three subgenera, including *Capillidium* Ben-Ze'ev & Kenneth, *Conidiobolus* Brefeld and *Delacroixia* Tyrrell & Macleod. Until 2018, no remarkable taxonomic treatments had been made for this genus, although additional species were reported continuously (Bałazy et al. 1987, Waters and Callaghan 1989, Bałazy 1993, Tosi et al. 2004, Huang et al. 2007, Waingankar et al. 2008, Nie et al. 2012, 2016, 2017, 2018). Meanwhile, higher-rank molecular phylogenetic studies on entomophthoroid fungi suggested *Conidiobolus* to be polyphyletic (Jensen et al. 1998, Gryganskyi et al. 2013, Nie et al. 2020). Consequently, the three genera *Capillidium*, *Microconidiobolus* and *Neoconidiobolus* were separated from *Conidiobolus* sensu lato and *Conidiobolus* sensu stricto was characterised by microspores arising from conidia (Nie et al. 2020).

During the past decade, Bo Huang's research group have carried out a comprehensive study on the taxonomy of *Conidiobolus* sensu lato in China and proposed five new species, five Chinese new records and 23 new combinations (Wang et al. 2010a, b, Nie et al. 2012, 2016, 2017, 2018, 2020, Chen and Huang 2018). Recent collections by this research group in eastern China resulted in the discovery of three unique species within the *Conidiobolus* sensu stricto lineage, which are described and illustrated herein with a multi-locus molecular phylogeny on the nuclear large subunit of rDNA (nucLSU), the mitochondrial small subunit of rDNA (mtSSU) and the translation elongation factor 1-alpha gene (TEF1).

## Materials and methods

### Isolates and morphology

Plant debris was collected from Taihushan and Jilongshan National Forest Parks, Anhui Province, China and Laoshan National Forest Park, Jiangsu Province, China. Isolations were carried out using the canopy-plating approach (Drechsler 1952, King 1976a). A Petri dish with potato dextrose agar (PDA; potato 200 g, dextrose 20 g, agar 20 g, H<sub>2</sub>O 1000 ml) was inverted over the plant debris and incubated at 21 °C



for daily examining for one week. When entomophthoroid fungi on the PDA canopy were detected, they were quickly transferred to new PDA and 2% water agar (agar 20 g, H<sub>2</sub>O 1000 ml) plates for purification and description. Morphological features were measured with an Olympus BX51 research microscope for 35 primary conidia and conidiophores each and photographed by an Olympus DP25 microscope-camera system. The descriptions were made with the method of King (1976a). Cultures were deposited in the Research Center for Entomogenous Fungi of Anhui Agricultural University, Anhui Province, China (RCEF) and the China General Microbiological Culture Collection Center, Beijing, China (CGMCC). Dried cultures were deposited in the Herbarium Mycologicum Academiae Sinicae, Beijing, China (HMAS). In order to infer the phylogeny of the genus *Conidiobolus* s.s., a total of 21 ex-types of species in *Conidiobolus* s.l., serving as outgroup, were obtained from the American Type Culture Collection, Manassas, USA (ATCC).

### DNA extraction, PCR amplification and sequencing

Fungal biomass was collected from the plate surface and ground in liquid nitrogen with a pestle and mortar. Genomic DNA was extracted using the CTAB method (Watanabe et al. 2010). The extracted DNA was stored in 100 µl TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) at -20 °C. Universal primer pairs LR0R (5'-ACC CGC TGA ACT TAA GC-3') and LR5 (5'-TCC TGA GGG AAA CTT CG-3') (Vilgalys and Hester 1990), mtSSU1 (5'-GCW GCA GTG RGG AAT NTT GGR CAA T-3') and mtSSU2R (5'-GTR GAC TAM TSR GGT ATC TAA TC-3') (Zoller et al. 1999) and EF983 (5'-GCY CCY GGH CAY CGT GAY TTY AT-3') and EF1aZ-1R (5'-ACA TCW CCG ACA CCC TTG ATC TTG -3') (Nie et al. 2012) were used for the amplification of the partial region of nucLSU, mtSSU and TEF1, respectively. The PCR reactions followed those in Liu et al. (2005) and Nie et al. (2012, 2020). A 50 µl mixture contained 200 µM dNTPs each, 1 × Mg-free buffer, 2.5 mM MgCl<sub>2</sub>, 0.5 µM primers each, 50 ng genomic DNA and 2 U Taq polymerase (Super Pfx DNA Polymerase, Cowinbioscience Co. Ltd., Shanghai, China). The programme consisted of an initial denaturation at 100 °C for 5 min without Taq polymerase, an extra denaturation at 95 °C for 5 min after the Taq polymerase was added, then 34 cycles of 94 °C for 1 min plus 55/54/57 °C (nucLSU / mtSSU / TEF1) for 2 min plus 72 °C for 2 min and a final extension at 72 °C for 10 min. The amplification products were sequenced by Shanghai GeneCore BioTechnologies Co. Ltd. (Shanghai, China), with the same primers as used in relative PCR reactions. All sequences were assembled with BioEdit (Hall 1999) and deposited at GenBank (Table 1).

### Phylogenetic analyses

In addition to the sequences obtained in this paper, nucLSU, mtSSU and TEF1 sequences of 20 strains in *Conidiobolus* sensu stricto were downloaded from GenBank.



**Table 1.** The taxa used in phylogenetic analyses.

Species	Strains*	GenBank accession numbers			References
		nucLSU	EF-1 $\alpha$	mtSSU	
<i>Capillidium adiaeretum</i>	CGMCC 3.15888	MN061284	MN061481	MN061287	Nie et al. 2020
<i>Ca. lobatum</i>	ATCC 18153 (T)	JF816218	JF816233	MK301187	Nie et al. 2012, 2020
<b><i>Conidiobolus bifurcatus</i> sp. nov.</b>	<b>CGMCC 3.15889 (T)</b>	<b>MN061285</b>	<b>MN061482</b>	<b>MN061288</b>	This article
<i>C. brefeldianus</i>	ARSEF 452 (T)	EF392382	–	EF392495	Genbank
<i>C. chlamydosporus</i>	ATCC 12242 (T)	JF816212	JF816234	MK301178	Nie et al. 2012, 2020
<i>C. coronatus</i>	NRRL 28638	AY546691	DQ275337	–	Lutzoni et al. 2004
<i>C. coronatus</i>	RCEF 4518	JN131537	JN131543	–	Nie et al. 2016, 2018
<i>C. dabieshanensis</i>	CGMCC 3.15763 (T)	KY398125	KY402206	MK301180	Nie et al. 2017, 2020
<i>C. firmipilleus</i>	ARSEF 6384	JX242592	–	JX242632	Gryganskyi et al. 2012
<i>C. gonimodes</i>	ATCC 14445 (T)	JF816221	JF816226	MK301182	Nie et al. 2012, 2020
<i>C. humicolus</i>	ATCC 28849 (T)	JF816220	JF816231	MK301184	Nie et al. 2012, 2020
<i>C. incongruus</i>	NRRL 28636	AF113457	–	–	Voigt et al. 1999
<i>C. iuxtagenitus</i>	ARSEF 6378 (T)	KC788410	–	–	Gryganskyi et al. 2013
<i>C. khandalensis</i>	ATCC 15162 (T)	KX686994	KY402204	MK301185	Nie et al. 2012, 2020
<i>C. lamprauges</i>	ARSEF 2338	DQ364206	–	DQ364226	Genbank
<i>C. lichenicolus</i>	ATCC 16200 (T)	JF816216	JF816232	MK301186	Nie et al. 2012, 2020
<i>C. macrosporus</i>	ATCC 16578 (T)	KY398124	KY402209	MK301188	Nie et al. 2017, 2020
<i>C. megalotocus</i>	ATCC 28854 (T)	MF616383	MF616385	MK301189	Nie et al. 2018, 2020
<i>C. mycophagus</i>	ATCC 16201 (T)	JX946694	JX946698	MK301190	Nie et al. 2018, 2020
<i>C. mycophilus</i>	ATCC 16199 (T)	KX686995	KY402205	MK301191	Nie et al. 2016, 2020
<i>C. parvus</i>	ATCC 14634 (T)	KX752051	KY402207	MK301192	Nie et al. 2016, 2020
<i>C. polyspermus</i>	ATCC 14444 (T)	MF616382	MF616384	MK301193	Nie et al. 2018, 2020
<i>C. polytocus</i>	ATCC 12244 (T)	JF816213	JF816227	MK301194	Nie et al. 2012, 2020
<b><i>C. taihushanensis</i> sp. nov.</b>	<b>CGMCC 3.16016 (T)</b>	<b>MT250086</b>	<b>MT274290</b>	<b>MT250088</b>	This article
<b><i>C. variabilis</i> sp. nov.</b>	<b>CGMCC 3.16015 (T)</b>	<b>MT250085</b>	<b>MT274289</b>	<b>MT250087</b>	This article
<i>Microconidiobolus nodosus</i>	ATCC 16577 (T)	JF816217	JF816235	MK333388	Nie et al. 2012, 2020
<i>M. terrestris</i>	ATCC 16198 (T)	KX752050	KY402208	MK301199	Nie et al. 2016, 2020
<i>Neoconidiobolus stromioideus</i>	ATCC 15430 (T)	JF816219	JF816229	MK301198	Nie et al. 2012, 2020
<i>N. thromboides</i>	ATCC 12587 (T)	JF816214	JF816230	MK301200	Nie et al. 2012, 2020

\*ARSEF, ARS Entomopathogenic Fungus Collection (Ithaca, U.S.A.). ATCC, American Type Culture Collection (Manassas, U.S.A). CGMCC, China General Microbiological Culture Collection Center (Beijing, China). NRRL, ARS Culture Collection (Peoria, U.S.A). RCEF, Research Center for Entomogenous Fungi (Hefei, China). T = ex-type.

Three genera *Capillidium*, *Microconidiobolus* and *Neoconidiobolus*, each represented by two species, were selected as outgroups. The nucLSU, mtSSU and TEF1 sequences were aligned with Clustal X (Thompson et al. 1997) and deposited at TreeBase (submission ID 26063). Phylogenetic analyses with Bayesian Inference (BI), Maximum Parsimony (MP) and Maximum Likelihood (ML) were carried out according to Nie et al. (2018, 2020). BI phylogeny was estimated using MrBayes 3.1.2 (Ronquist and Huelsenbeck 2003). The best-fit model selected with the Akaike Information Criterion (AIC) in Modeltest 3.7 (Posada and Crandall 1998) was used to evaluate Posterior Probabilities (PP) and the critical value for the topological convergence diagnostic was set to 0.01 of the average standard deviation of split frequencies. Four Markov chains ran simultaneously from random starting trees for 0.5 million generations and trees were sampled every 100<sup>th</sup> generation. MP analyses were performed using a heuristic search with PAUP\* 4.0b10 (Swofford 2002). All characters were weighted and gaps were treated as missing data. Tree bisection-reconnection (TBR) was set as the branch swapping algo-



rithm. Branch robustness was estimated with bootstrapping 1,000 replicates (Felsenstein 1985). ML analyses were performed with the RAxML (Stamatakis 2006), implemented in raxmlGUI 1.5b1 (Silvestro and Michalak 2012). Branch reliabilities were determined by 1,000 ML rapid bootstrap replicates with the GTRGAMMA substitution model. Phylogenetic trees were checked and modified in FigTree 1.4 (Rambaut 2012).

## Results

### Phylogenetic analyses

The combined nucLSU+TEF1+mtSSU dataset was composed of 29 taxa representing 27 species and 1949 characters including 986 constant, 276 parsimony-uninformative and 687 parsimony-informative. The most parsimonious tree was generated with a tree length (TL) of 2716 steps, a consistency index (CI) of 0.5497, a homoplasy index (HI) of 0.4503, a retention index (RI) of 0.6191 and a rescaled consistency index (RC) of 0.3403. The best model applied in the BI analysis was GTR+I+G. The final average standard deviation of split frequencies was 0.0086 and the final likelihood value was -14423. The three phylograms resulted in similar topologies and the ML tree was presented along with MP/ML bootstrap and BI posterior probability values at relative branches (Fig. 1).

Three clades can be seen to form for the *Conidiobolus* s.s. The three species, described here, were located in clade I.

### Taxonomy

#### *Conidiobolus bifurcatus* B. Huang & Y. Nie, sp. nov.

MycoBank No: 831599

Facesoffungi: FoF 08142

Figure 2

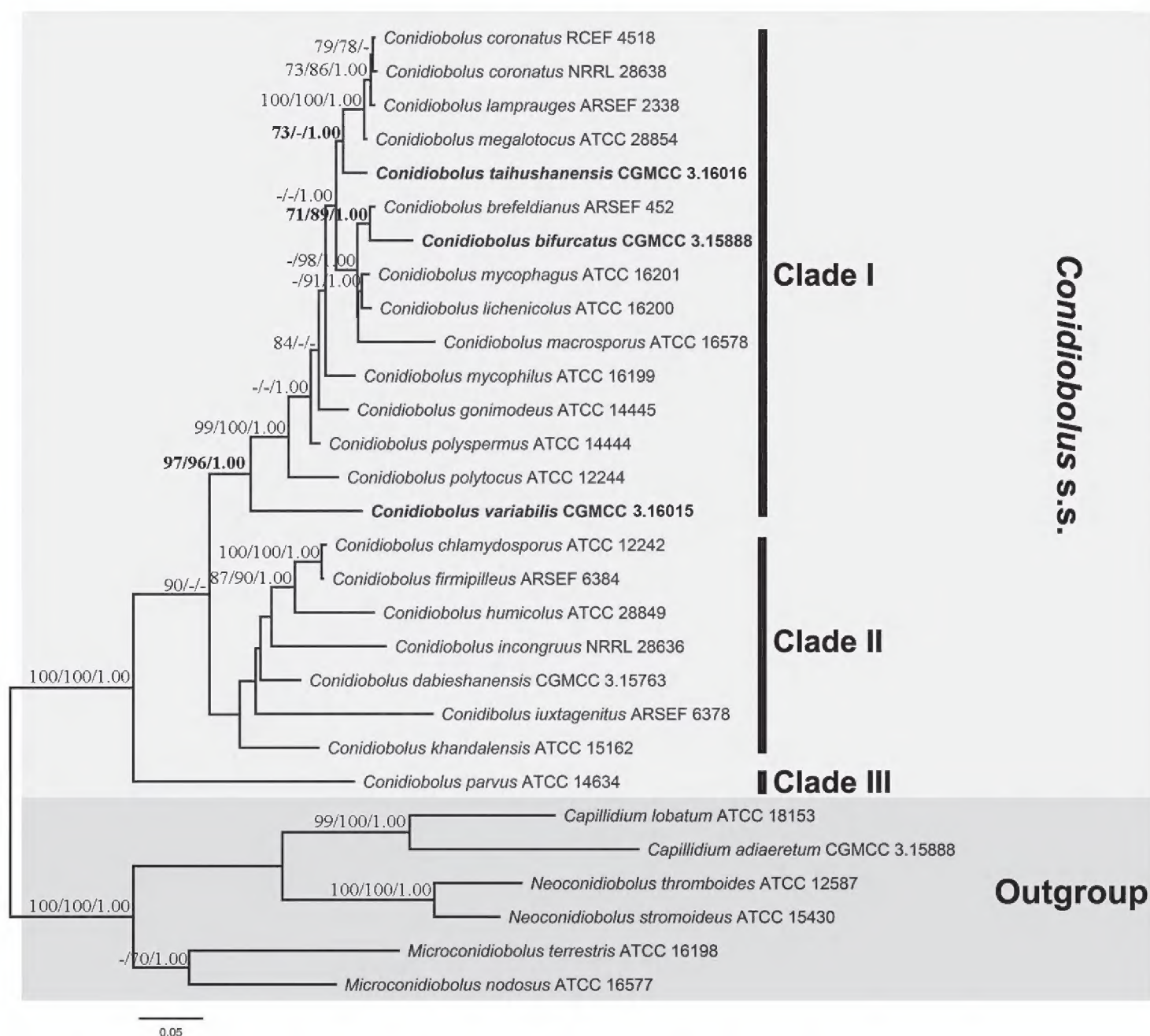
**Typification.** CHINA, Jiangsu: Nanjing, Laoshan National Forest Park, 32°6'7"N, 118°36'17"E, from plant debris, 1 Dec 2018, Y. Nie and Y. Gao (holotype HMAS 248359, ex-holotype culture CGMCC 3.15889 = RCEF 6551, GenBank: nucLSU = MN061285; TEF1 = MN061482; mtSSU = MN061288).

**Etymology.** *bifurcatus* (Lat.), referring to secondary conidiophores often branched at the tip to form two short stipes, each bearing a secondary conidium.

**Ecology and distribution.** Plant debris in Jiangsu Province, China.

**Description.** Colonies on PDA at 21 °C for 3 d, opaque, white, reaching ca. 2 mm in diameter, with many small colonies around the periphery due to discharged conidia. Mycelia colourless, 8–11 µm wide, rarely branched and non-septate when young, often septate and distended to a width of 10–27 µm after 5 d. Primary conidiophores arising from the hyphal segments, colourless, 38–254 × 7.5–12 µm, unbranched and producing a single globose conidium, without widening upwards near the tip. Primary conidia for-



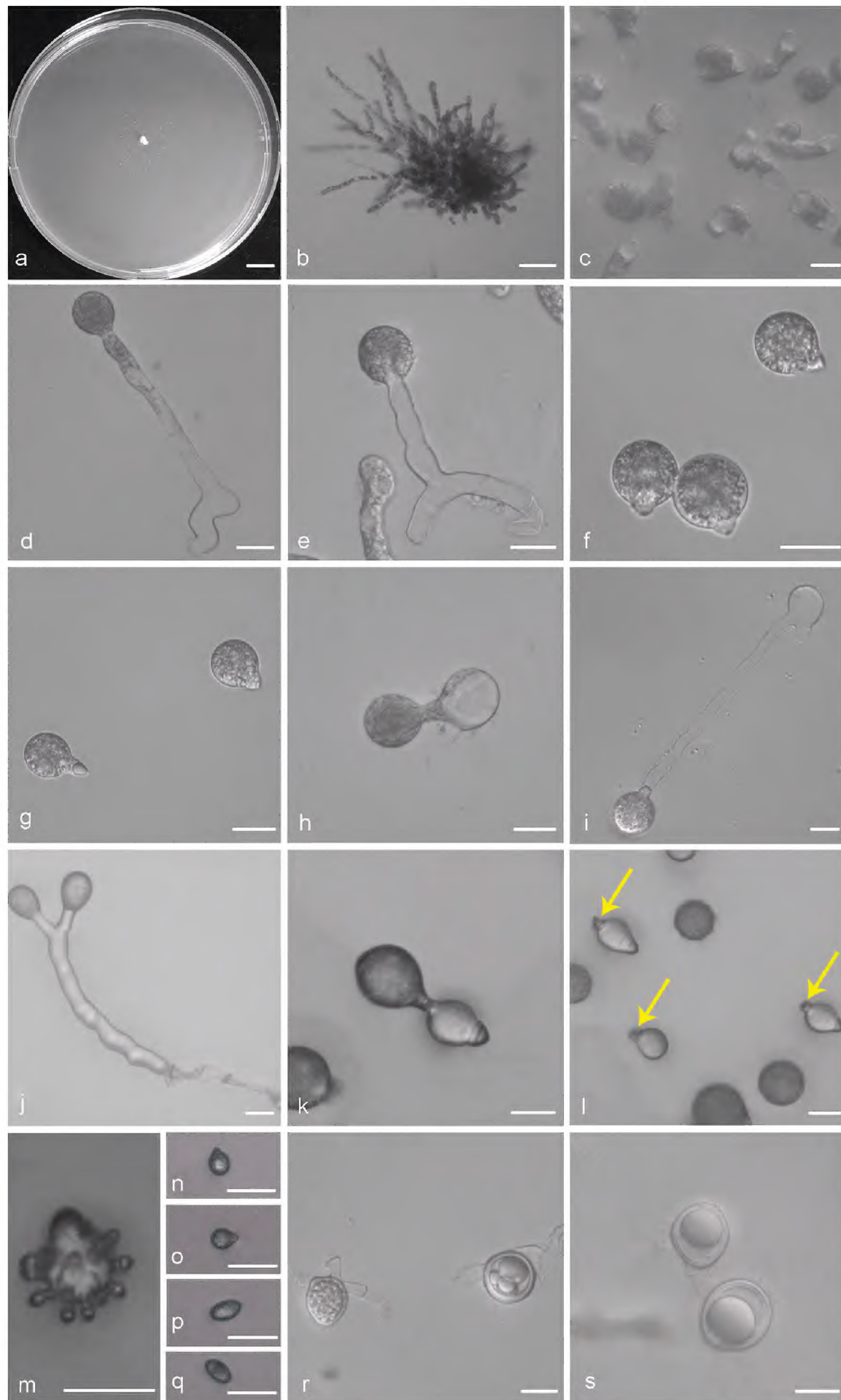


**Figure 1.** Phylogenetic tree of *Conidiobolus* s.s. reconstructed by maximum likelihood analyses of nucLSU, mtSSU and TEF1 sequences, with six *Conidiobolus* s.l. species as outgroups. Three new species of *Conidiobolus* are shown in bold. Maximum parsimony bootstrap values ( $\geq 70\%$ ) / Maximum likelihood bootstrap values ( $\geq 70\%$ ) / Bayesian posterior probabilities ( $\geq 0.95$ ) of each node are indicated along branches. Scale bar indicates substitutions per site.

cibly discharged, globose to subglobose,  $2\text{--}40 \times 2\text{--}33 \mu\text{m}$ , with a papilla more or less tapering and pointed,  $7\text{--}11 \mu\text{m}$  wide at the base,  $3\text{--}12 \mu\text{m}$  long. Secondary conidiophores arising from the primary conidia, often branched almost at the tip, forming two short stipes each bearing a secondary conidium. Secondary conidia similar to, but smaller than the primary ones, mostly forcibly discharged, occasionally falling off and leaving a relic of the secondary conidiophores. On 2 % water agar, microconidia produced readily, globose to ellipsoidal,  $7\text{--}12 \times 6\text{--}9 \mu\text{m}$ . Zygosporangia homothallic, usually formed between adjacent segments of the same hypha after an incubation of 5–7 d at  $21^\circ\text{C}$  on PDA, smooth, mostly globose,  $25\text{--}40 \mu\text{m}$  in diameter, with a  $1.5\text{--}3 \mu\text{m}$  thick wall.

**Notes.** *Conidiobolus bifurcatus* sp. nov. is characterised by its secondary conidiophores, which are often bifurcated near the tip and bear a secondary conidium on each





**Figure 2.** *Conidiobolus bifurcatus* sp. nov. **a** Colony on PDA after 3 d at 21 °C **b** mycelium **c** septate mycelium and distended segments **d, e** primary conidiophores bearing primary conidia **f, g** primary conidia **h, i** a single secondary conidium produced from primary conidia **j** two secondary conidia arising from a branched conidiophore **k** secondary conidia falling from primary conidia **l** the relic of secondary conidiophores on secondary conidia (arrows) **m** microconidia arising from a conidium **n, o** globose microconidia **p, q** ellipsoidal microconidia **r** zygospores formed between adjacent segments of the same hypha **s** zygospores. Scale bars: 10 mm (**a**); 100 µm (**b**); 20 µm (**c–s**).



stipe. Morphologically, it is allied to *Conidiobolus mycophilus* Srin. & Thirum., which has smaller primary conidia (Srinivasan and Thirumalachar 1965). It appears to be similar to *C. incongruus* Drechsler and *C. mycophagus* Srin. & Thirum. in the size of primary conidia and zygospores and the formation of microconidia, but different in its longer primary conidiophores (Drechsler 1960; Srinivasan and Thirumalachar 1965). However, it is distantly related to these two species in the molecular phylogenetic tree. Instead, it is phylogenetically closely related to *C. brefeldianus* Couch (Figure 1: MP 71/ML 89/BI 1.00), but morphologically distinct by its larger primary conidia and zygospores (Couch 1939).

***Conidiobolus taihushanensis* B. Huang & Y. Nie, sp. nov.**

MycoBank No: 835124

Facesoffungi: FoF 08143

Figure 3

**Typification.** CHINA, Anhui: Ma'anshan City, Hanshan County, Taihushan National Forest Park, 31°30'53"N, 118°2'49"E, from plant detritus, 12 Jan 2019, *Y. Nie and Y. Cai* (holotype HMAS 248724, ex-holotype culture CGMCC 3.16016 = RCEF 6559, GenBank: nucLSU = MT250086; TEF1 = MT274290; mtSSU = MT250088).

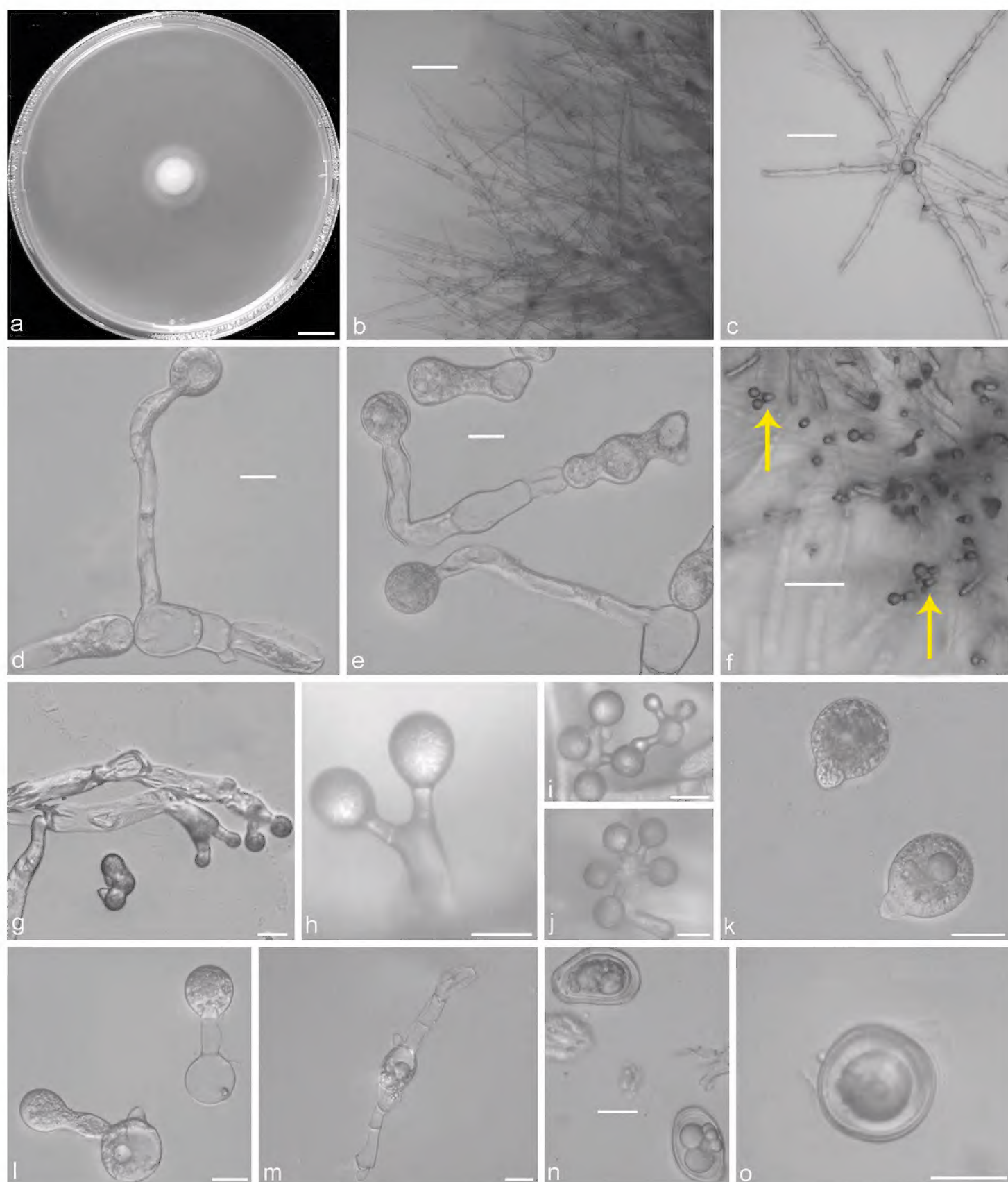
**Etymology.** *taihushanensis* (Lat.), referring to the region where the fungus was isolated.

**Ecology and distribution.** Plant debris in Anhui Province, China.

**Description.** Colonies on PDA at 21 °C after 3 d, white, reaching ca. 11–14 mm in diameter. Mycelia colourless, straight and unbranched when young, 8.5–12 µm wide; distended and non-contiguously segmented when old, 10–20 µm wide. Primary conidiophores arising from the older mycelia without an upward widening near the tip, colourless, 44–180 × 7–13 µm, usually unbranched and often producing a single globose primary conidium, at the initial growth stage 2–5 short branches bearing a primary conidium each. Primary conidia forcibly discharged, mostly subglobose, 27–42 × 19–32 µm, with tapering and pointed papilla, 4–10 × 8–12 µm. Secondary conidia arising from primary conidia, similar to, but smaller than the primary ones, forcibly discharged. On 2% water agar, microconidia not observed. Zygospores usually formed between adjacent segments of the same hypha after 5 d, 34–48 × 23–40 µm, with a 2–4 µm thick wall, ellipsoid and rich in content when young, smooth, mostly globose, subglobose to ovate when mature.

**Notes.** *Conidiobolus taihushanensis* sp. nov. is morphologically highly distinct with its straight apical mycelia and the production of 2–5 conidia from the hyphal body. *Conidiobolus taihushanensis* sp. nov. is similar to *C. polytocus* Drechsler in the structure of several short branches at the top of conidiophores, but the latter is distinguished by smaller primary conidia (12–25 × 14–29 µm) and slightly curved mycelia (Drechsler 1955c). *Conidiobolus taihushanensis* sp. nov. is related to *C. margaritatus* B. Huang, Humber & K.T. Hodge and *C. megalotocus* Drechsler by the size of primary conidia, but *C. margaritatus* forms a chain of undischarged repetitional conidia (Huang et al. 2007) and *C. megalotocus* lacks zygospores (Drechsler 1956). Phylogenetically, *C. taihushanensis* sp. nov. is closely related to *C. megalotocus* (Figure 1: MP 73/BI 1.00) and distantly related to *C. polytocus*, though no molecular data are available for *C. margaritatus*. Phylogenetically, *C. taihushanensis* sp.





**Figure 3.** *Conidiobolus taihushanensis* sp. nov. **a** colony on PDA after 3 d at 21 °C **b** mycelia unbranched at the colony edge **c** young mycelia **d, e** primary conidiophores arising from mycelia segments **f** two branches germinated from hyphal bodies and each bearing a primary conidium (arrows) **g–j** two, three, four or five branches germinated from hyphal bodies and each bearing a primary conidium **k** globose to subglobose primary conidia **l** secondary conidia arising from primary conidia **m** zygosporangia formed between adjacent segments of the same hypha **n** young zygosporangia **o** mature zygosporangia. Scale bars: 10 mm(**a**); 100 µm (**b, c, f**); 20 µm (**d, e, g–o**).

nov. is also closely related to *C. lamprauges* Drechsler and *C. coronatus* Batko, but it differs from *C. lamprauges* by larger primary conidia ( $27\text{--}42 \times 19\text{--}32\text{ }\mu\text{m}$  vs.  $12.5\text{--}20 \times 15\text{--}22\text{ }\mu\text{m}$ ) and from *C. coronatus* by the absence of villose resting spores (Drechsler 1953a).



***Conidiobolus variabilis* B. Huang & Y. Nie, sp. nov.**

MycoBank No: 835125

Facesoffungi: FoF 08144

Figure 4

**Typification.** CHINA, Anhui: Ma'anshan City, Hexian County, Jilongshan National Forest Park, 31°48'1"N, 118°12'19"E, from plant debris, 23 Dec 2017, Y. Nie (holotype HMAS 248723, ex-holotype culture CGMCC 3.16015 (= RCEF 6540), GenBank: nucLSU = MT250085; TEF1 = MT274289; mtSSU = MT250087).

**Etymology.** *variabilis* (Lat.), referring to producing various shapes of primary conidia.

**Ecology and distribution.** Plant debris from Anhui Province, China.

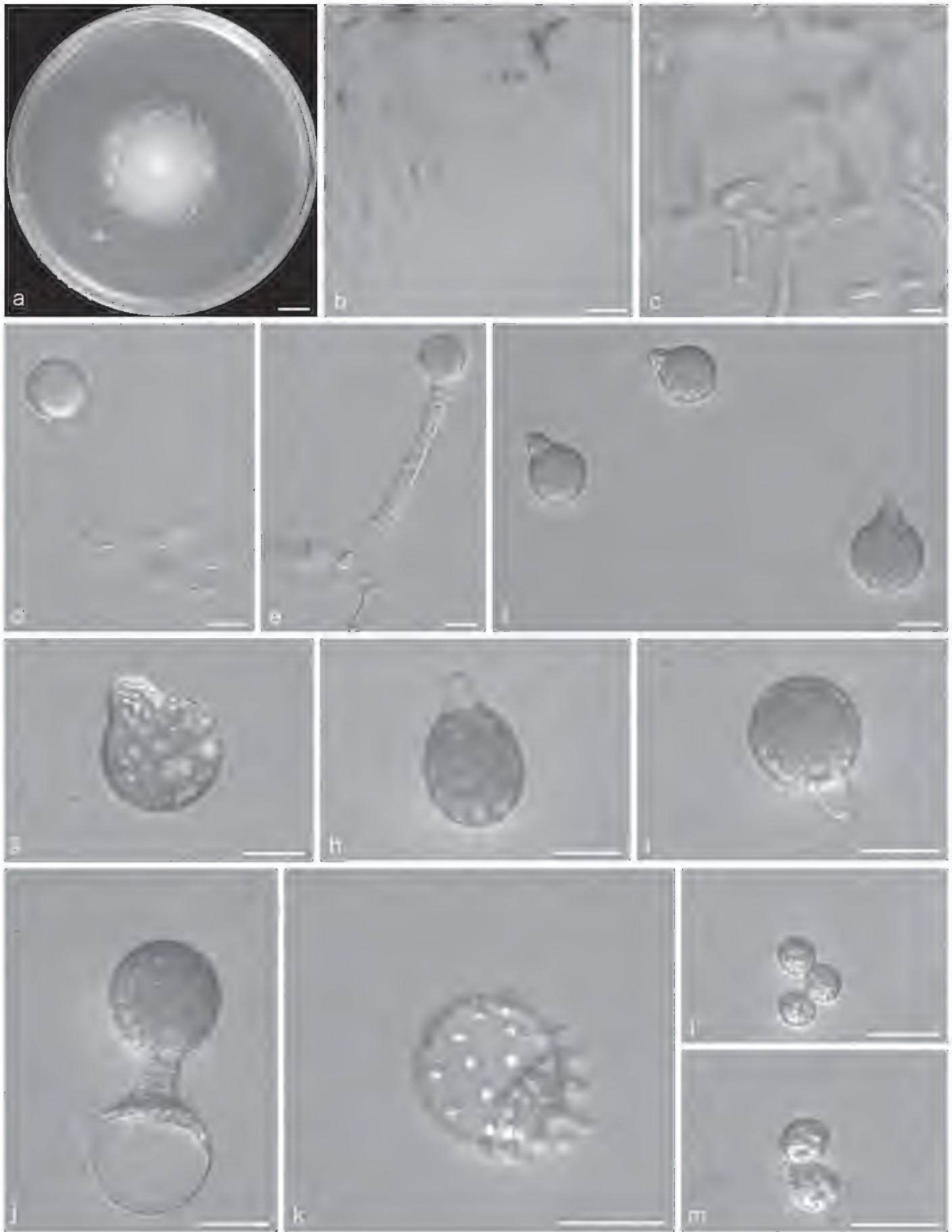
**Description.** Colonies on PDA at 21 °C after 3 d white, reaching ca. 41–48 mm in diameter. Mycelia colourless, 6–11 µm wide, rarely branched at the colony edge. Primary conidiophores unbranched and producing a single globose conidium, colourless, 60–200 × 9–15 µm, without an upward widening near the tip. Primary conidia forcibly discharged, globose, subglobose, pyriform to obovoid, 31–55 × 25–40 µm, with tapering and pointed papilla, 3.5–9 × 8–13 µm. Secondary conidia arising from primary conidia, similar to, but smaller than primary ones, forcibly discharged. On 2% water agar, microconidia rarely observed, globose, subglobose to ellipsoidal, 10–12 × 9–14 µm. Resting spores not observed.

**Notes.** Considering the large size of primary conidia, *Conidiobolus variabilis* sp. nov. is allied to *C. coronatus* (Cost.) Batko (14.5–38.5 × 17–48.5 µm), *C. macrosporus* Srin. & Thirum. (38–45 × 48–54 µm) and *C. utriculosus* Brefeld (25–35 × 37.5–51 µm). It is distinguished from *C. coronatus* by its various shapes of primary conidia and the absence of villose spores. It differs from *C. macrosporus* by its longer primary conidiophores and the absence of resting spores (Batko 1964, Srinivasan and Thirumalachar 1967). It is differentiated from *C. utriculosus* by the shapes of primary conidia and the absence of zygosporangia. Phylogenetically, *C. variabilis* sp. nov. is basal in clade I and distantly related to *C. coronatus* and *C. macrosporus*.

## Discussion

The genus *Conidiobolus* has recently been divided into four lineages and one of them was treated as *Conidiobolus* sensu stricto on the basis of a synapomorph, namely microspores (Nie et al. 2020). The three new species *C. bifurcatus* sp. nov., *C. taihushanensis* sp. nov. and *C. variabilis* sp. nov. are located in the clade of *Conidiobolus* s.s. (Fig. 1). *Conidiobolus taihushanensis* sp. nov. was paraphyletic to *C. megalotocus* Drechsler, *C. lamprauges* Drechsler and *C. coronatus* (Cost.) Batko with a robust support of BI posterior probability of 1.00. *Conidiobolus bifurcatus* sp. nov. was a sister group to *C. brefeldianus*, which was supported by all three inferring methods (MP 71/ML 89/BI 1.00). *Conidiobolus variabilis* sp. nov. was basal in clade I with a relatively high confidence (MP 97/ML 96/BI 1.00). *Conidiobolus bifurcatus* sp. nov. and *C. variabilis* sp. nov.





**Figure 4.** *Conidiobolus variabilis* sp. nov. **a** Colony on PDA after 3 d at 21 °C **b** mycelia rarely branched at the colony edge **c** mycelia **d,e** primary conidiophores bearing primary conidia **f–i** primary conidia with different shapes **j** secondary conidia arising from primary conidia **k** microconidia arising from conidia **l** globose microconidia **m** ellipsoidal microconidia. Scale bars: 10 mm (**a**); 100 µm (**b**); 20 µm (**c–m**).

morphologically produce microspores. However, *C. taihushanensis* sp. nov. lacks this synapomorph. Besides *C. taihushanensis* sp. nov., four other species in the *Conidiobolus* s.s., i.e. *C. dabieshanensis* Y. Nie & B. Huang, *C. iuxtagenitus* S.D. Waters & Calla-



ghan, *C. lamprauges* and *C. parvus* Drechsler were not reported to produce microspores either. This may be due to the need for particular conditions, such as growth temperature and nutritional supply. For example, the microspores of *C. khandalensis* Srin. & Thirum. were only observed on 2% water-agar at 16 °C (Nie et al. 2020).

Except microspores, species of the *Conidiobolus* s.s. clade are morphologically diverse, particularly the secondary conidia. For instance, *C. iuxtagenitus* produces single fusiform discharged secondary conidia (Waters and Callaghan 1989) and *C. margaritatus* forms a necklace-like chain of up to seven undischarged conidia (Huang et al. 2007). Although these special characteristics provide good identification, most members of this lineage are difficult to distinguish phenotypically. Sequence data of nucLSU and TEF1 have provided a better understanding of species circumscription or inter- and intraspecific variations (Nie et al. 2012). In this study, morphology and molecular data support *C. bifurcatus* sp. nov., *C. taihushanensis* sp. nov. and *C. variabilis* sp. nov. as new species in the *Conidiobolus* s.s. clade. Although the microspores of *C. taihushanensis* sp. nov. were not observed, its straight apical mycelium and the production of 2–5 conidia from the hyphal body make it easily distinguishable from other species of *Conidiobolus* s.s.

With the proposal of the three new species herein, 17 species are currently accepted in the genus *Conidiobolus* s.s. and only five were found distributed in China (King 1976a, b, 1977, Wang et al. 2010a, b, Nie et al. 2017, 2020). For updating, the key to all these 17 species are provided as follows.

### Key to the species of *Conidiobolus* s.s.

- |   |   |                                      |
|---|---|--------------------------------------|
| 1 | Villose resting spores produced .....   | <b><i>Conidiobolus coronatus</i></b> |
| – | Villose resting spores not produced .....   | 2                                    |
| 2 | Microspores produced .....  | 3                                    |
| – | Microspores not observed .....  | 4                                    |
| 3 | Two types of sexual reproduction, zygosporangia formed in axial alignment with one or both conjugating segments ..... | 5                                    |
| – | One type of sexual reproduction, zygosporangia formed in one of the conjugating segments .....                        | 6                                    |
| 5 | Primary conidia larger, up to 51 µm .....   | <b><i>C. utriculosus</i></b>         |
| – | Primary conidia smaller, less than 36 µm .....  | <b><i>C. brefeldianus</i></b>        |
| 6 | 2–4 branches germinated at the top of primary conidiophores .....   | 7                                    |
| – | Unbranched at the top of conidiophores .....  | 8                                    |
| 7 | Only 2 primary conidia arising from 2 branches, larger, up to 44 µm .....   | <b><i>C. megalotocus</i></b>         |
| – | 2–4 primary conidia arising from 2–4 branches, smaller, less than 29 µm .....   | <b><i>C. polytocus</i></b>           |
| 8 | Secondary conidiophores branched .....  | 9                                    |
| – | Secondary conidiophores unbranched .....  | 10                                   |



- 9 Secondary conidiophores branched almost at the tip, primary conidia larger, up to 40  $\mu\text{m}$ ..... *C. bifurcatus* sp. nov.
- Secondary conidiophores branched at the tip or base, primary conidia smaller, less than 30  $\mu\text{m}$  ..... *C. mycophilus*
- 10 Primary conidia larger, up to 55  $\mu\text{m}$  ..... 11
- Primary conidia smaller, maximum not over 42  $\mu\text{m}$ ..... 12
- 11 Primary conidia globose to pyriform, zygosporangia globose, 26–40  $\mu\text{m}$ ..... *C. macrosporus*
- Primary conidia globose, subglobose, pyriform to obovoid, zygosporangia not observed ..... *C. variabilis* sp. nov.
- 12 Primary conidia smaller, less than 21  $\mu\text{m}$  ..... *C. khandalensis*
- Primary conidia larger, more than 33  $\mu\text{m}$ ..... 13
- 13 Two types of resting spores produced: zygosporangia or chlamydospores..... *C. humicolus*
- One type of resting spores produced ..... 14
- 14 Only chlamydospores produced..... *C. firmipilleus*
- Only zygosporangia produced ..... 15
- 15 Primary conidiophores shorter, less than 80  $\mu\text{m}$ ..... *C. gonimodes*
- Primary conidiophores longer, more than 130  $\mu\text{m}$  ..... 16
- 16 Zygosporangia globose or elongate, larger, 15–40  $\times$  18–45  $\mu\text{m}$  ... *C. incongruus*
- Zygosporangia globose, smaller, 30–36  $\mu\text{m}$  ..... *C. mycophagus*
- 4 Fusiform secondary conidia produced, each zygosporangium in a position separated by a short, but relatively constant distance from a lateral conjugation outgrowth or beak..... *C. iuxtagenitus*
- Fusiform secondary conidia not produced, each zygosporangium in a position not separated by a short, but relatively constant distance from a lateral conjugation outgrowth or beak ..... 17
- 17 A chain of up to seven undischarged repetitional conidia produced ..... *C. margaritatus*
- No chains of undischarged repetitional conidia produced ..... 18
- 18 Primary conidiophores produced from cushion mycelia..... *C. lichenicolus*
- Primary conidiophores not produced from cushion mycelia..... 19
- 19 Apical mycelia straight, 2–5 conidia arising from hyphal body, no chlamydospores, zygosporangia produced..... *C. taihushanensis* sp. nov.
- Apical mycelia slightly curved, unbranched at the top of conidiophore, chlamydospores produced, no zygosporangia..... *C. dabieshanensis*

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